UNIVERSITY OF MINNESOTA

Twin Cities Campus

Division of Epidemiology
School of Public Health

Suite 300
1300 South Second Street
Minneapolis, MN 55454-1015
612-624-1818
Fax: 612-624-0315

October 13, 1999

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

RE: Food Labeling; Health Claims and Label Statements,
Federal Register, Sept. 8, 1999, Docket Nos. 91N-0101,
91N-0098, 91N-0103, 91N-100H

This letter is in response to a request for scientific data related to a proposed health claim on 0.8 mg folic acid from supplements vs. food. We published results (see enclosed paper) that directly addresses this issue. It is clear that diets containing folate and folic acid from fortified foods are sufficient to provide protection from neural tube defects. The 0.8 mg folic acid proposed is excessive and supplements are not required to achieve the desired level of protection. The 0.8 mg level is higher than recently released Reference Dietary Intakes from the National Academy of Sciences. These recommendations call for consumption of 600 dietary folate equivalents per day during pregnancy. The claim proposed should not be allowed on scientific grounds.

Sincerely,

Judith E. Brown, PhD, MPH, RD
Professor, Public Health Nutrition

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nmol/L (141 and 228 ng/mL) in women with pregnancies affected by NTDs vs 517 and 630 nmol/L (228 and 278 ng/mL), respectively, in women whose pregnancies were not affected by NTDs. Of the 12 cases of NTDs identified in these 2 studies, only 1 maternal red cell folate value exceeded 680 nmol/L (300 ng/mL). Lack of data on the effect of folate intake on red cell folate levels in women of childbearing age currently limits our ability to provide practical guides on folate intake.[25]

This study sought to identify predictors of red cell folate level and relationships between folate intake and red cell folate levels in women attempting to become pregnant. Since the bioavailability of naturally occurring folates in food (principally present as pteroylpolyglutamates) and folic acid (pteroylmonoglutamate) used in fortified breakfast cereals and supplements differs,[26] we also sought to identify levels of red cell folate associated with consumption of the different sources of folate. Results are used to estimate levels of folate intake that correspond to reduced risk of NTDs.

METHODS

Study participants consisted of a subset of women in the Diana Project, a prospective study of preconceptional and prenatal risks to reproductive outcomes. (The study was named after the Roman goddess Diana. In classical mythology, Diana was a goddess of childbirth and the moon; she was intimately concerned with the affairs of women.) Participants for the Diana Project were recruited by mail between 1989 and 1992 from the entire population of women between the ages of 22 to 35 years in Group Health, Inc, a large health maintenance organization (HMO) serving the greater Minneapolis-St Paul, Minn, area. Data collection was completed by mid 1994. Women were eligible for the Diana Project if they were intending to become pregnant during the enrollment period, had no history of chronic disease or infertility, and would not be using contraceptive methods during enrollment. We estimate that 1 in 4 eligible women participated. The Diana sample was remarkably similar to the HMO's population of women 22 to 35 years of age. Variables such as infant birth weight, gestational age, race, pregnancy weight gain, income, and educational level were similar among participants and nonparticipants. Notably, participants were less likely to smoke cigarettes (7.2%) than nonparticipants (13.2%). Additional information on the study's methods and sample characteristics and the representativeness of the sample is available.[27]

The current study used the subset of 219 Diana Project participants sequentially selected to receive red cell folate and serum ferritin and zinc analyses in addition to hemoglobin (which was routinely tested on all participants) and had completed 1 or more food frequency questionnaires (FFQs) around the time of the blood draw. Women in this substudy were attempting to become pregnant or were within their first month of pregnancy when blood was drawn. To determine if women in this substudy differed in sociodemographic or other characteristics from women in the larger study, characteristics of women in the Diana Project who had completed 1 or more FFQs prior to 30 days after conception but had not had the additional laboratory tests run were compared with characteristics of women included in this study. All women provided written consent; the study was approved by the appropriate institutional review boards.

Information on weight, parity, menstrual cycles, dietary intake, vitamin and mineral supplement use, oral contraceptive use, smoking status, race, household income, educational level, and occupation was collected by mailed questionnaires. Conception was based on a positive pregnancy test; days postconception were primarily based on monthly records of menstrual cycles. Height and laboratory values were assessed during a Diana Project study visit.

Dietary intake was assessed by the Willett FFQ[28] modified to reflect dietary intake during the previous month. The FFQ was completed monthly prior to conception for 4 months and then every 3 months if conception did not occur. It was completed monthly during pregnancy. "Other foods" listed by participants in response to the question "Are there any other important foods that you usually eat at least once per week?" were coded and entered into the nutrient analyses. Completed FFQs were edited in standard fashion and scanned by the Data Recognition Corporation, Minnetonka, Minn.

All of the 219 women in the study had completed an FFQ within 2 months before or 1 month after the blood draw. In addition, 172 had completed a second FFQ within 3 months before the one nearest to the blood draw. The mean ± SD number of days between the 2 FFQs was 36.7 ± 13.4. Depending on availability, 1 FFQ or average results of 2 FFQs (weighted by 2) were used in the multivariate models. Previous results of comparisons of dietary intake assessed by 4-day, weighed food records among a subset of 56 preconceptional women in the Diana Project showed a favorable correlation (r=0.67) between total folate intake assessed by the FFQ and the 4-day record.[29] The folic acid content of fortified cereals was estimated as 100 μg per cup (240 mL), the serving size listed on the FFQ. Food folate was estimated as total folate minus folic acid from fortified cereals. Cereals were the only folic acid–fortified food reported by women in this study.

Intake of vitamins and minerals in supplements was assessed monthly by a detailed questionnaire that solicited information on the brand name, type, frequency of use, and dose levels of supplements taken. Updated information on vitamin and mineral supplement contents by brand name from the Nutrition Coordinating Center at the University of Minnesota and other resources were used to identify and verify the contents of the supplements listed by participants.

Nonfasting venous blood samples were drawn into trace element–free vacutainers and vacutainers containing ethylenediaminetetraacetic acid (EDTA) or heparin. Red cell folate was preserved by the addition of 0.4% ascorbate solution, and levels were determined by standard radioimmunoassay procedures (Quantaphase IR Folate Radioassay, Bio-Rad Laboratories, Mississippi, Ontario). The laboratory's coefficient of variation (CV) for red cell folate was 9.7%. Serum zinc was determined by flame atomic-absorption spectrophotometry; the CV was 9.5% for the zinc measurements.

Pearson correlations between variables were examined to screen covariates and candidates for entry into the regression models. Red cell folate values were log transformed to normalize the distribution. Regression models were developed for the full sample and for nonusers of folic acid supplements. The models included biologically pertinent variables and those with a Pearson correlation coefficient with red cell folate of $P<.1$. In situations where intakes of specific nutrients were observed to be potentially related to red cell folate but covaried with other nutrients, each nutrient was entered into the regression model and assessed for its contribution to R^2. Those not contributing or contributing the least to the model were deleted. Additional models incorporating curvature in the association of folate intake with red cell folate and nutrient interaction terms (eg, folic acid and iron supplements) were tested. Final models used 5% 2-sided tests. Version 6.09 of the SAS program (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Women in this study were similar in age, race, employment status, occupational category, educational and income

Table 1.—Laboratory Values, Dietary and Supplement Intake, and Other Characteristics of the 219 Study Participants

Characteristic	Mean (SE) or %
Laboratory values	
Red cell folate, nmol/L [ng/mL]	772 (23) [340 (10)]
Serum zinc, μmol/L [μg/dL]	15 (0.3) [98 (2)]
Dietary intake	
Folate, μg/d	255 (9)
Iron, mg/d	11.9 (0.4)
Zinc, mg/d	12.1 (0.3)
Vitamin C, mg/d	126.4 (5.2)
Vitamin B_{12}, μg/d	6.0 (0.2)
Alcohol, g/d	3.4 (0.3)
Breakfast cereal users, % yes	86
No. of servings per week by users	3.2 (0.2)
Folic acid–fortified cereal users, % yes	74
No. of servings per week by users	3.4 (0.2)
Vitamin/mineral supplements	
Users of supplements, % yes	35
Users of folic acid, % yes	27
Dose of folic acid, μg/d	339 (37)
Users of iron, % yes	28
Dose of iron, mg/d	22 (3)
Users of zinc, % yes	25
Dose of zinc, mg/d	14 (2)
Users of vitamin C, % yes	32
Dose of vitamin C, mg/d	269 (65)
Smokers, % yes	12
Oral contraceptive use within past year, % yes	22

Table 2.—Coefficient Estimates of Variables Associated With Red Cell Folate Level Among the Total Sample and Among Nonusers of Folic Acid Supplements

Variables	Total Sample, PE (95% CI)* (n=189)	Nonusers of Folic Acid Supplements, PE (95% CI)* (n=133)
Folic acid supplement, mg/d	0.30 (0.20 to 0.40)	...
Folic acid from fortified cereals, g/d	1.28 (0.72 to 1.83)	1.57 (0.94 to 2.21)
Vitamin C supplement, g/d	0.10 (0.03 to 0.17)	0.09 (0.01 to 0.18)
Serum zinc, nmol/L [mg/dL]	−163.71 (−247.86 to −79.56) [−1.07 (−1.62 to −0.52)]	−100.52 (−84.46 to 285.50) [−0.66 (−0.55 to 1.87)]
Intercept	2.52 (2.42 to 2.62) R^2=0.29	2.45 (2.32 to 2.58) R^2=0.19

*Parameter estimate (95% confidence interval).

level, and body mass index to preconceptional women in the Diana Project who did not have the additional laboratory tests (data not shown). Groups differed in parity ($P<.05$), but parity was found not to be related to red cell folate level in the regression models. Characterization of sociodemographic status, dietary intakes, vitamin and mineral supplement use, and laboratory values of women in the current study is shown in Table 1.

Variables entered into regression models consisted of age, occupation, education, and income categories; use of oral contraceptives within the past year; parity; body mass index; energy intake and dietary intake of iron, zinc, vitamin C, vitamin B_{12}, alcohol, folate from foods, and folic acid from fortified cereals; amount of supplemental folic acid, iron, zinc, and vitamin C; and serum zinc and ferritin levels. Values for each of these measures were available for 189 women. Results of regression analyses of predictors of red cell folate in the total sample led to the exclusion of all variables except folic acid supplements, folic acid intake from fortified cereals, vitamin C supplement dose, and serum zinc (inversely related) in the final model (Table 2). When only women who did not take folic acid supplements were included in the analyses, serum zinc dropped from the model.

The Figure shows results obtained when relationships between folate intake and red cell folate levels were plotted by the level of intake among women consuming the different sources of folate. The relationship between folate intake and red cell folate level among the 41 women in the sample whose sole source of folate was that naturally occurring in foods increased from the lowest to the second quartile ($P=.002$), but did not increase further through the third and fourth quartile of food folate intake. Red cell folate level increased as cereal and supplemental sources of folic acid were added, reaching a plateau at the higher levels of folic acid supplementation.

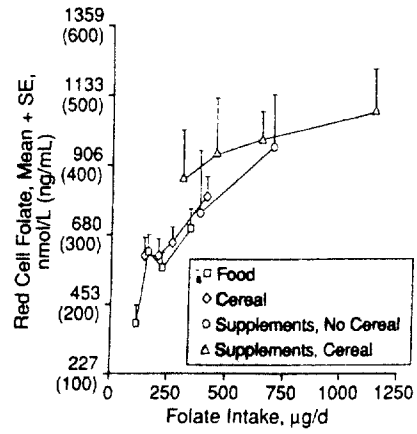
Interactive effects of combinations of supplemental iron, dietary iron, supplemental vitamin C, dietary vitamin C, dietary folate, supplemental folic acid, dietary zinc, and supplemental zinc on red cell folate level were tested in regression models. Of these, the interaction of supplemental iron with supplemental folic acid was negatively related to red cell folate level ($P=.002$, multiple $r=0.32$). This model correctly fitted the flattened association seen in the Figure at high supplemental folic acid intakes. A competing model that included the square of folic acid supplements and excluded iron supplements yielded the same goodness of fit (multiple $r=0.32$).

Examinations of correlates of serum zinc revealed a lack of correlation with dietary or supplemental intake of iron, zinc, or folate; or dietary fiber, caffeine, or vitamin C intake. An interactive effect of iron and folic acid supplements on serum zinc was not identified.

Mean folate intakes and red cell folate levels varied by fortified cereal and supplement use (data not shown). Women consuming only naturally occurring sources of folate had the lowest mean±SE folate intake (213±18 μg/d) and red cell folate level (616±39 nmol/L [272±17 ng/mL]). Women who consumed folic acid–fortified cereals in addition to naturally occurring sources of folate had a significantly higher ($P<.05$) mean±SE folate intake (260±14 μg/d) and red cell folate level (712±27 nmol/L [314±12 ng/mL]) than women who consumed only naturally occurring sources of folate. Folic acid supplement users had the highest mean±SE intake of folate (613±52 μg/d) and red cell folate level (983±65 nmol/L [434±29 ng/mL]).

COMMENT

After accounting for the potential effects of a number of sociodemographic, lifestyle, dietary, and other exposures on red cell folate level, this study identified folic acid supplements, folic acid–fortified cereals, vitamin C supplements, and serum zinc level (inverse) as being independently associated with red cell folate level. Among nonusers of folic acid supplements, folic acid–fortified cereals and vitamin C supplements were independently related to red cell folate level. Although intake of naturally occurring sources of folate was not independently related to red cell folate level across all intake levels, a significant correlation ($P=.002$) between intake of naturally occurring folates and red cell folate level was observed in women within the lowest half of the intake distribution. Measurement error in red cell folate analyses, determinations of the folate composition of food, the assessment of folate intake, and variations in the bioavailability of folate consumed may have led to misclassification of women and a bias toward the null. Because the half-life of folate in red blood cells is approximately 100 days,[30] it is possible that differences in timing between folate consumption and red cell folate tests altered relationships somewhat. Even given these potential limitations, this study of community-living women may more closely reflect relationships between usual food and supplement intake practices and red cell folate levels than feeding studies conducted under tightly controlled circumstances.



Red cell folate level by geometric mean folate intake within groups defined by sources of folate. "Food" values represent quartile intakes of folate from foods by 41 women not consuming folic acid–fortified cereals or folic acid supplements; "cereal" values represent quartiles of folate intake from foods plus folic acid–fortified cereal by 115 women consuming folic acid–fortified cereal and not taking folic acid supplements; "supplement, no cereal" values represent the median split of folate intakes from foods plus supplements among 13 nonconsumers of fortified cereal; and "supplements, cereal" values represent quartiles of folate intake from foods, fortified cereal, plus supplements among 47 folic acid supplement users.

The relationship of supplemental folic acid intake and red cell folate level was not linear, as shown by the plateau in red cell folate at the highest levels of supplemental folic acid intake. We tested a number of nutrient interactions that may account for this effect and found that the interaction of supplemental iron with supplemental folic acid was negatively related to red cell folate level. Two regression models described the plateau in the red cell folate curve equally well. One fitted folic acid from supplements as a quadratic curve, the other fitted an iron supplement by folic acid supplement interaction. If the first model were biologically correct, it would imply that red cells were saturated with folate; but others[3,31] have observed higher levels of red cell folate than we identified here. We suggest there may be an antagonism between supplemental folic acid and supplemental iron in regard to the level of red cell folate in this sample of women attempting pregnancy. It is also possible that the flattening of the red cell folate curve at higher levels of folate intake is related to a decreased availability of folic acid when taken with food[32,33] or to other, unmeasured factors.

Vitamin C supplements were found to be independently related to red cell folate level in the multivariate models used for the full sample and for nonusers of supplemental folic acid. Vitamin C may enhance red cell folate level by protecting tetrahydrofolic acid from oxidation.[26] Interestingly, Smithells et al[18] identified significantly higher levels of white blood cell vitamin C as well as red cell folate in the first trimester of pregnancy in women whose pregnancies were unaffected by NTDs compared with women whose pregnancies were.

We are unable to explain the independent, inverse association between serum zinc and red cell folate level in supplement users. This finding is of concern, however, since studies have associated high plasma folate and low serum zinc levels with pregnancy complications and adverse outcomes.[34-37] Although a number of studies have concluded that folic acid supplements interfere with zinc absorption or utilization,[38-40] other results have led to the opposite conclusion.[41-43] It is possible that unmeasured factors related to supplement use or supplement users account for this effect.

Red cell folate levels of less than 453 nmol/L (200 ng/mL), which are indicative of a negative folate balance,[44] were identified in 1 in 8 women. Nearly half of the women (44%) had red cell folate levels less than 680 nmol/L (300 ng/mL), while only 1 in 4 had red cell folate levels higher than 906 nmol/L (400 ng/mL). These figures indicate that folate insufficiency is common among this group of primarily well-educated, middle-income women. Of the 710 pregnancies followed in the Diana Project, 2 cases of NTDs were identified. Unfortunately, red cell folate levels are not available for these pregnancies.

Results of the studies reported by Laurence et al[3] and Smithells and coworkers[18] in which red cell folate levels were assessed prior to the second trimester of pregnancy suggest that red cell folate levels in excess of 680 nmol/L (300 ng/mL) are highly protective against NTD. Evidence provided by the larger study of Daly and colleagues[25] indicates that red cell folate levels higher than 906 nmol/L (400 ng/mL) may be optimal. Red cell folate levels were determined in this latter study at a median of 15 weeks' gestation and may be different than at the time of neural tube closure. We suspect that red cell folate levels reported by Daly et al[25] may be higher than levels at neural tube closure (approximately 21 days' gestation) owing to the use of prenatal supplements containing folic acid and vitamin C.

Mean intakes of folate related to mean red cell folate levels exceeding 680 nmol/L (300 ng/mL) in this study ranged from 309 to 411 µg per day depending on the sources of folate. Women who consumed approximately 1 serving of folic acid–fortified cereal per day on average (100 µg of folic acid) in addition to an average intake of 231±38 µg of folate per day from naturally occurring folates had a mean±SE red cell folate level of 750 ± 77 nmol/L (331 ± 34 ng/mL). Red cell folate levels higher than 906 nmol/L (400 ng/mL) were nearly exclusively found among supplement users in the current study. Extrapolating from the data presented in the Figure, it appears that folate intakes of more than 500 µg per day from foods and fortified cereals are needed to achieve mean red cell folate levels in excess of 906 nmol/L (400 ng/mL) in nonusers of folic acid supplements. Folate intakes of 500 µg per day could be achieved by the consumption of vegetables, fruits, and folic acid–fortified cereals. In this study, 1 serving of vegetables or fruit contributed an average of 42 µg of folate. Consumption of 5 vegetables and fruits a day and 1 serving of a cereal, such as Product 19 or Total fortified with 400 µg of folic acid per serving, would theoretically result in folate intakes over 500 µg. Among all women consuming folic acid supplements, a mean intake of approximately 450 µg of folate per day is related to mean red cell folate levels higher than 906 nmol/L (400 ng/mL). Daily use of a 400-µg folic acid supplement would result in red cell folate levels that exceed 906 nmol/L (400 ng/mL) in a majority of women.

Characteristics of women in the Diana Project are similar to those of women in the HMO from which the sample was drawn. However, the HMO and the sample include a very low proportion of women from minority groups, and most women were in the middle-income group. Consequently, results identified here will best apply to middle-income, white women.

The forthcoming fortification in the United States of refined cereal and grain products with 140 µg of folic acid per 100 g is expected to increase folate intake by a minimum of approximately 80 µg per day.[45] Since the mean folate intake of women of childbearing age in the United States is around 206 µg per day,[46] fortification of refined cereals and grains would elevate mean folate intake to at least 286 µg per day. Based on results presented in the Figure, this minimal level of folate intake from foods and fortified products would theoretically result in a minimal, mean red cell folate level of approximately 634 nmol/L (280 ng/mL). The extent to which folic acid fortification of refined cereal and grain products protects against folate-responsive NTDs will depend on whether levels of red cell folate higher than 680 or 906 nmol/L (300 or 400 ng/mL) correspond to the prevention of abnormal neu-

ral tube closure. Nonetheless, these data indicate that folic acid fortification of cereal and grain products will not provide optimal protection against folate-responsive NTDs.

While fortification of refined cereal and grain products with folic acid will play an important role in the prevention of NTDs, population-based strategies and interventions for those at high risk will be needed to reduce the incidence of NTDs given the high prevalence of folate insufficiency. Educational activities, such as broad-based campaigns focused on the need for high-folate foods, folic acid–fortified products, and folic acid supplements before and very early in pregnancy, and easy access to folic acid supplements, will be needed to fill in the folate gap among women with low folate intakes.

This research was supported by grant HD19724 from the National Institute of Child Health and Human Development.

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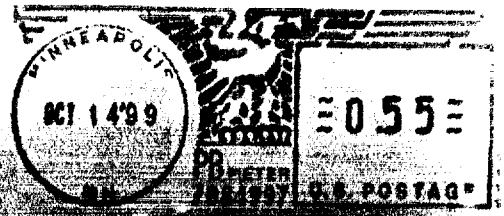
HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CROSS REFERENCE SHEET

Docket Number/Item Code:	91N-0101/C172
See Docket Number/Item Code:	91N-0098/C118 91N-0103/C105 91N-100H/C97



UNIVERSITY OF MINNESOTA

Twin Cities Campus
Dr. Judith E. Brown
Division of Epidemiology
School of Public Health
[illegible]

Dockets Management Branch (HFA-305)
Food and Drug Administration
[illegible]
Rockville, MD 20852